embryonic kidney epithelial cells which are competent to replicate these adenoviral vectors (293 cells). The vector incorporating the SMA promoter demonstrated substantial selectivity for vascular smooth muscle gene expression, with typical transductions carried out in parallel under identical conditions manifesting 90–95% lacZ-expressing BASMC, 0.3% lacZ-positive A549 cells, and 4% positive 293 cells. Conversely, parallel transductions with the vector employing the RSV promoter typically resulted in 95–99% lacZ-expressing 293 cells at vector concentrations yielding only 5–10% positive BASMC. These data support cell lineage-specificity of AvLacZ5 at the level of promoter function rather than due to intrinsic cellular differences in capacity for adenovirally-mediated transduction. However, it is notable that a limited subpopulation of 293 cells clearly are able to direct sufficient transcription from the SMA promoter sequences chosen to yield detectable lacZ expression; the molecular basis for this heterogeneity of expression remains to be determined. Adenoviral vectors utilizing these promoter sequences may render vascular-restricted gene transfer feasible when used in conjunction with mechanical devices providing a component of spatial localization.

1012-102 Alteration of Rabbit Carotid Artery Vasomotor Function by Gene Transfer with a Replication Deficient Adenovirus

Brian H. Annex, Mark G. Davies, Keith M. Channon, Michael A. Blazing, Doris A. Taylor, Samuel E. George, Stephen M. Denning, Per-Otto Hagen, Kevin G. Peters. *Division of Cardiology and Experimental Surgery, Duke University Medical Center, Durham, NC*

Gene transfer technologies offer great potential both to investigate and alter the course of vessel wall pathophysiology. Replication-deficient adenovirus vectors appear particularly useful for the transfection of blood vessels, because, of their ability to accommodate large cDNA inserts and to rapidly and efficiently infect mammalian cells. However, the potential effects of transfection with a replication-deficient adenovirus on vasomotor function have not been previously described. We performed gene transfer experiments with a replication-deficient adenovirus which contained a cytomegalovirus promoter and a nuclear-localizing variant of the bacterial β-galactosidase gene. Excised carotid artery segments from 6 rabbits were divided into multiple segments and infected in pairs, for 30 minutes with four different viral titers (0, 2.5×10^9, 5×10^9, 1×10^{10} pfu/ml) at 37°C in serum free media. These segments were tested for β-galactosidase staining and vasomotor function 2 days later. Isometric tension studies were performed to examine the response of the vessel segments to the contractile agonist, norepinephrine (NE). The results are shown below as mean ± standard error, $-\log_{10}[EC_{50}]$ with * $p \leq 0.04$ between the lowest and the highest group.

	Viral Titer (pfu/ml)			
	0	2.5×10^9	5×10^9	1×10^{10}
NE Dose	5.98 ± 0.09	5.77 ± 11	5.68 ± 0.14	5.52 ± 0.13*

*$p \leq 0.04$

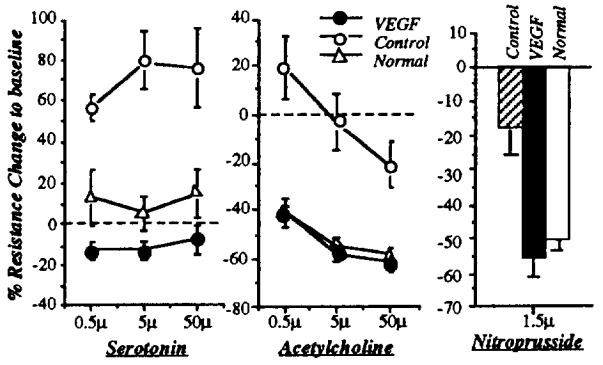
In cell culture, rabbit vascular smooth muscle cells were infected in a similar manner. β-Galactosidase staining was present at all 3 viral concentrations but when a viral concentration of 1×10^{10} pfu/ml was used there were areas of cellular necrosis not seen at the lower viral concentrations.

Conclusion: Replication-deficient adenovirus vectors can rapidly and efficiently infect rabbit carotid vessels. However at doses often employed for gene transfer experiments *in-vivo*, there is a dose dependent alteration of vessel vasomotor function which may be mediated by a cytotoxic affect of the viral vector. This effect needs to be taken into account in studies involving adenoviral gene transfer.

1012-103 Restoration of Endothelial Function in Hypercholesterolemic Rabbit by Intermittent Administration of Vascular Endothelial Growth Factor

Takayuki Asahara, Christophe Bauters, Tiangen Wu, Lu P. Zheng, Donghui Chen, Marianne Kearney, Susan Rossow, Stuart Bunting, Napoleone Ferrara, James F. Symes, Jeffrey M. Isner. *St. Elizabeth's Medical Center, Boston, MA; Genentech Inc., South San Francisco, CA*

Endothelial dysfunction in hypercholesterolemia causes abnormal arterial vasoreactivity and precedes the onset of atherosclerosis. Vascular endothelial growth factor (VEGF) is an endothelial cell (EC) specific mitogen that has been shown to stimulate EC proliferation in vitro and in vivo. We investigated the hypothesis that VEGF could also modulate EC dysfunction and thereby improve endothelium-dependent vasoreactivity, impaired due to hypercholesterolemia. Hypercholesterolemic rabbits fed a 1% cholesterol diet for 6 weeks were treated with intravenous administration of 300 μg VEGF (VEGF group; n = 8) or 0.9% saline solution (control group; n = 9) twice a week for 3 weeks, during which time both groups continued to receive cholesterol diet. All rabbits were evaluated by in vivo vasomotion study; 8 normal (non-cholesterol and non-treated) rabbits were evaluated as well. The vasoreactive response to acetylcholine, serotonin and nitroprusside were calculated from flow velocity measured by Doppler wire, vessel diameter obtained from angiograms, and intra-arterial blood pressure recorded at the proximal external iliac artery. The resistance response to endothelium-dependent and -independent agonists recovered in VEGF group, as illustrated below. Furthermore, average intimal thickness of external iliac artery and lower abdominal aorta in VEGF group was significantly less than control group (VEGF = 0.010 ± 0.008 vs control = 0.106 ± 0.036, $p < 0.05$). *Conclusion:* Intermittent systemic administration of VEGF improves endothelial dysfunction and attenuates intimal thickness in hypercholesterolemic rabbit.



1012-104 Monoclonal Antibody to Tissue Factor Inhibits Intravascular Thrombosis without Impairing Extravascular Hemostasis

Claude R. Benedict, Jerry Todd, Aruna B. Pawashe, Michael D. Ezekowitz. *University of Texas Medical School, Houston, TX; Yale University Medical School, New Haven, CT*

Background: Recent studies suggest that the release of tissue factor (TF) and its interaction with factor VII at the site of vascular injury may be the activator of the coagulation cascade, which along with platelet aggregation leads to occlusive intravascular thrombus formation. We tested this hypothesis in a rabbit carotid artery model of thrombosis.

Methods: Carotid artery was instrumented with Doppler flow probe and a needle electrode. Partially occlusive thrombus was formed by applying 150 μA of current which damages the endothelium of the carotid artery and promotes thrombus formation. After development of 50% occlusion of the artery by thrombus, the current was stopped and a murine monoclonal antibody against rabbit TF (AP-1) (0.1 mg/kg intraarterially) or vehicle (control) was administered. The changes in carotid blood flow were continuously monitored by the Doppler flow probe. Bleeding was assessed by weighing the amount of blood absorbed in a preweighed sponge, placed in a cut wound that was 5 cm long and 0.5 cm deep.

Results: The control rabbits (n = 12) occluded their arteries in 46.2 ± 13.6 min after stopping the current by a fibrin-platelet thrombus. In contrast, AP-1 prevented carotid artery occlusion for >200 min (n = 6) ($p > 0.0001$). Lower doses of AP-1 were ineffective. The deep incisional bleeding times were not different between the control animals and the treated group. Similarly the platelet counts or *ex vivo* platelet aggregation response to collagen, ADP or arachidoic acid were not different between the control and the treated animals.

Conclusion: Data suggest that TF released at the site of vascular injury plays a role in intravascular coagulation and its activity can be selectively inhibited, without inducing an alteration in hemostatic parameters or platelet functions.

1012-105 Endothelin Immunoreactivity in Human Coronary Atherosclerosis

Amir Lerman, Kirk N. Garratt, David R. Holmes Jr., William D. Edwards, Linda J. McKinley, John C. Burnett Jr.. *Mayo Clinic, Rochester, MN*

Coronary atherosclerosis and restenosis are disease processes involving cell proliferation and activation of growth factors. Endothelin-1 (ET-1), a vasoconstrictor and mitogenic peptide, is produced from its precursor Big-ET. Coronary plasma ET concentrations are elevated in coronary atherosclerosis and following coronary interventions. The current study was designed to test the hypothesis that ET is produced and present in human coronary atherosclerotic and restenotic lesions and localized to both endothelial and nonendothelial cells. Twenty coronary lesions were obtained utilizing directional atherectomy. Immunohistochemistry for endothelins was performed with rabbit polyclonal ET-1 and Big-ET antiserum without cross reactivity be-